

Supporting Information

To

Single-Molecule Study of Metalloregulator CueR–DNA Interactions Using Engineered Holliday Junctions

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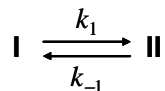
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I. Derivation of the single-molecule kinetics of the structural dynamics of HJC2

A. Free HJC2

The structural dynamics of a HJ, if measured at the single-molecule level at tens of milliseconds time resolution, follows a two-state kinetics effectively:



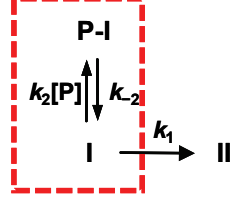
where I denotes conf-I and II denotes conf-II (see also Figure 1 in the main text). The waiting time τ_I in the E_{FRET} trajectories is the time needed to complete I \rightarrow II transition; the waiting time τ_{II} is to complete II \rightarrow I transition; both are simple one-step kinetic reactions. The probability density functions for τ_I and τ_{II} , $f_I(\tau)$ and $f_{II}(\tau)$, are both single-exponential functions, with $f_I(\tau) = k_1 \exp(-k_1 \tau)$ and $f_{II}(\tau) = k_{-1} \exp(-k_{-1} \tau)$. The inverse of the average waiting times, $\langle \tau_I \rangle^{-1}$ and $\langle \tau_{II} \rangle^{-1}$, which represent the time-averaged single-molecule rates of I \rightarrow II and II \rightarrow I transitions respectively, are:

$$\langle \tau_I \rangle^{-1} = \frac{1}{\int_0^{\infty} \tau f_I(\tau) d\tau} = k_1 \quad (\text{A1})$$

$$\langle \tau_{II} \rangle^{-1} = \frac{1}{\int_0^{\infty} \tau f_{II}(\tau) d\tau} = k_{-1} \quad (\text{A2})$$

B. Apo-CueR and HJC2 interactions

The kinetic mechanism of apo-CueR interactions with HJC2 is shown in Figure 5A. The kinetic processes happening during τ_I are the following kinetic steps:



The corresponding single-molecule rate equations are:

$$dP_{II}(t)/dt = k_1 P_I(t) \quad (\text{B1})$$

$$dP_I(t)/dt = -(k_1 + k_2[P])P_I(t) + k_{-2}P_{P-I}(t) \quad (\text{B2})$$

$$dP_{P-I}(t)/dt = k_2[P]P_I(t) - k_{-2}P_{P-I}(t) \quad (\text{B3})$$

where $P(t)$'s are the probabilities of finding HJC2 in the corresponding states at time t and k 's are the rate constants for the transitions. At the on-set of each τ_1 , i.e., right after a $II \rightarrow I$ transition, the first state that HJC2 reaches is I; so the initial conditions for solving the above differential equations are: $P_I(0) = 1$, $P_{II}(0) = 0$, $P_{P-I}(0) = 0$, where $t = 0$ being the on-set of each τ_1 . And at any time, $P_I(t) + P_{II}(t) + P_{P-I}(t) = 1$.

We can then evaluate the probability density function of τ_1 , $f_1(\tau)$. The probability of finding a particular τ is $f_1(\tau)\Delta\tau$, which is equal to the probability for HJC2 to switch from I to II between τ and $\tau + \Delta\tau$, $\Delta P_{II}(\tau)$ (1, 2). Therefore, $f_1(\tau)\Delta\tau = \Delta P_{II}(\tau)$. In the limit of infinitesimal $\Delta\tau$, $f_1(\tau)$ is equal to $dP_{II}(\tau)/d\tau$. Solving for $P_{II}(\tau)$ using equations B1-B3 by Laplace transform, the probability density function of τ_1 is:

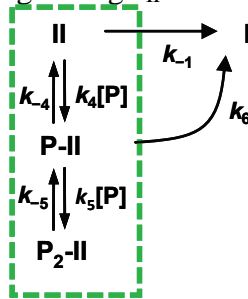
$$f_1(\tau) = \frac{k_1 e^{(\alpha+\beta)\tau}}{2\alpha} [\alpha(1 + e^{-2\alpha\tau}) + (\beta + k_{-2})(1 - e^{-2\alpha\tau})]$$

where $\alpha = -\sqrt{\frac{1}{4}(k_1 + k_{-2} + k_2[P])^2 - k_1 k_{-2}}$ and $\beta = -\frac{(k_1 + k_{-2} + k_2[P])}{2}$. Then:

$$\langle \tau_1 \rangle^{-1} = 1 / \int_0^\infty \mathcal{F}_1(\tau) d\tau = \frac{k_1}{1 + [P]/K_{P-I}}$$

where $K_{P-I} = k_{-2}/k_2$ is the dissociation constant for the apo-CueR-conf-I complex. This equation is given as Eq. 1 in the main text.

The kinetic processes happening during τ_{II} are the following kinetic steps:



The corresponding single-molecule rate equations are:

$$dP_I(t)/dt = k_{-1}P_{II}(t) + k_6P_{P-II}(t) \quad (B4)$$

$$dP_{II}(t)/dt = -(k_{-1} + k_4[P])P_{II}(t) + k_{-4}P_{P-II}(t) \quad (B5)$$

$$dP_{P-II}(t)/dt = k_4[P]P_{II}(t) - (k_{-4} + k_6 + k_5[P])P_{P-II}(t) + k_{-5}P_{P_2-II}(t) \quad (B6)$$

$$dP_{P_2-II}(t)/dt = k_5[P]P_{P-II}(t) - k_{-5}P_{P_2-II}(t) \quad (B7)$$

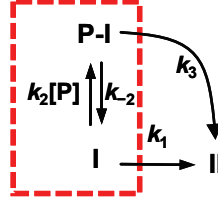
The initial conditions for solving above equations are: $P_{II}(0) = 1$, $P_I(0) = 0$, $P_{P-II}(0) = 0$, and $P_{P_2-II}(0) = 0$. And at any time, $P_I(t) + P_{II}(t) + P_{P-I}(t) + P_{P_2-II}(t) = 1$. Similarly, $f_{II}(\tau) = dP_I(\tau)/d\tau$. Using equations B4-B7 to solve for $P_I(\tau)$, we can obtain $f_{II}(\tau)$. Then,

$$\langle \tau_{II} \rangle^{-1} = 1 / \int_0^\infty \mathcal{F}_{II}(\tau) d\tau = \frac{k_{-1} + k_6[P] / K'_{P-II}}{1 + [P] / K'_{P-II} + [P]^2 / (K'_{P-II} K_{P_2-II})}$$

where $K'_{P-II} = (k_{-4} + k_6)/k_4$ and $K_{P_2-II} = k_{-5}/k_5$. This equation is given as Eq 2 in the main text.

C. Holo-CueR and HJC2 interactions.

The kinetic mechanism for holo-CueR–HJC2 interactions is shown in Figure 5B. The kinetic processes happening during τ_I are:



The corresponding single-molecule rate equations are:

$$dP_{II}(t)/dt = k_1P_I(t) + k_3P_{P-I}(t) \quad (C1)$$

$$dP_I(t)/dt = -(k_1 + k_2[P])P_I(t) + k_{-2}P_{P-I}(t) \quad (C2)$$

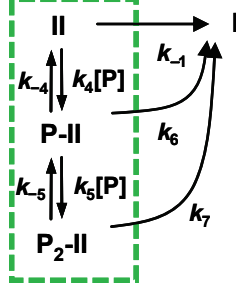
$$dP_{P-I}(t)/dt = k_2[P]P_I(t) - (k_{-2} + k_3)P_{P-I}(t) \quad (C3)$$

The initial conditions are $P_I(0) = 1$, $P_{II}(0) = 0$ and $P_{P-I}(0) = 0$ and at any time, $P_I(t) + P_{II}(t) + P_{P-I}(t) = 1$. Similarly, $f_I(\tau) = dP_{II}(\tau)/d\tau$, and solving equations C1-C3 for $P_{II}(\tau)$, we can obtain $f_I(\tau)$, and

$$\langle \tau_I \rangle^{-1} = 1 / \int_0^\infty \mathcal{F}_I(\tau) d\tau = \frac{k_1 + [P]k_3 / K'_{P-I}}{1 + [P] / K'_{P-I}}$$

where $K'_{P-I} = (k_{-2} + k_3)/k_2$. This equation is given as Eq. 3 in the main text.

The kinetic processes happening during τ_{II} are:



The corresponding single-molecule rate equations are:

$$dP_I(t)/dt = k_{-1}P_{II}(t) + k_6P_{P-II}(t) + k_7P_{P_2-II}(t) \quad (C4)$$

$$dP_{II}(t)/dt = -(k_{-1} + k_4[P])P_{II}(t) + k_{-4}P_{P-II}(t) \quad (C5)$$

$$dP_{P-II}(t)/dt = k_4[P]P_{II}(t) - (k_{-4} + k_6 + k_5[P])P_{P-II}(t) + k_{-5}P_{P_2-II}(t) \quad (C6)$$

$$dP_{P_2-II}(t)/dt = k_5[P]P_{P-II}(t) - (k_{-5} + k_7)P_{P_2-II}(t) \quad (C7)$$

The initial conditions for solving above equations are: $P_{II}(0) = 1$, $P_I(0) = 0$, $P_{P-II}(0) = 0$, and $P_{P_2-II}(0) = 0$. And at any time, $P_I(t) + P_{II}(t) + P_{P-II}(t) + P_{P_2-II}(t) = 1$. Similarly, $f_{II}(\tau) = dP_I(\tau)/d\tau$. Using equations C4-C7 to solve for $P_I(\tau)$, we can obtain $f_{II}(\tau)$ and $\langle \tau_{II} \rangle^{-1}$ for holo-CueR–HJC2 interactions.

Inconveniently, the expressions of the solutions to equations C4–C7 are so tediously complex to hamper their physical understanding. To get a clean analytical expression for $\langle \tau_{II} \rangle^{-1}$, we arbitrarily set $k_{-4} = 0$ and get:

$$\langle \tau_{II} \rangle^{-1} = \frac{k_{-1} + [P](k_{-1}k_7/(k_6K'_{P_2-II}) + k_6/K'_{P-II}) + [P]^2 k_7/(K'_{P-II}K'_{P_2-II})}{1 + [P](k_7/(k_6K'_{P_2-II}) + 1/K'_{P-II}) + [P]^2/(K'_{P-II}K'_{P_2-II})}$$

where $K'_{P-II} = k_6/k_4$ and $K'_{P_2-II} = (k_{-5} + k_7)/k_5$. This equation is given as Eq. 4 in the main text. As this equation can satisfactorily interpret the [holo-CueR] dependence of $\langle \tau_{II} \rangle^{-1}$, we use it to fit the holo-CueR data in Figure 4B to obtain other relevant kinetic parameters.

Reference

1. Xie, X. S. 2001. Single-molecule approach to enzymology. *Single Mol.* 2:229-236.
2. Xu, W., J. S. Kong, and P. Chen. Single-molecule kinetic theory of heterogeneous and enzyme catalysis. *J. Phys. Chem. C.* in press.

Supporting Figures

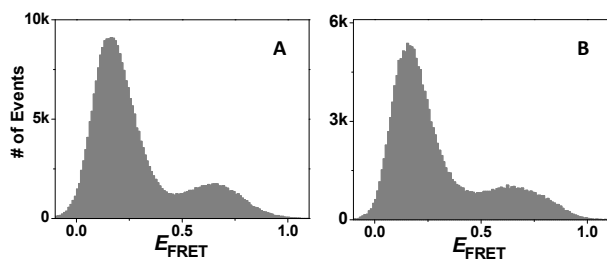


Figure S1. Histograms of HJC2 E_{FRET} trajectories in the absence (A) and presence of $1.0 \mu\text{M}$ apo-PbrR691 (B). Bin size: 0.01. Each histogram is compiled from more than 100 trajectories

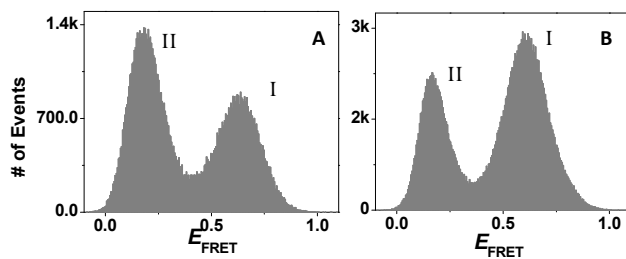


Figure S2. Histograms of HJC2 E_{FRET} trajectories in the presence of $0.5 \mu\text{M}$ apo-CueR (A) and $3 \mu\text{M}$ apo-CueR (B). Bin size: 0.005.

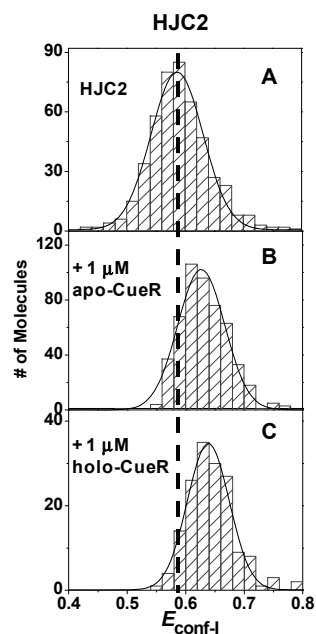


Figure S3. Histograms of $E_{\text{conf-I}}$ of HJC2 (A) in the presence of 1 μM apo-CueR (B) and 1 μM holo-CueR (C). Solid lines are Gaussian fits centered at 0.59 ± 0.01 (A), 0.63 ± 0.01 (B), and 0.64 ± 0.01 (C).

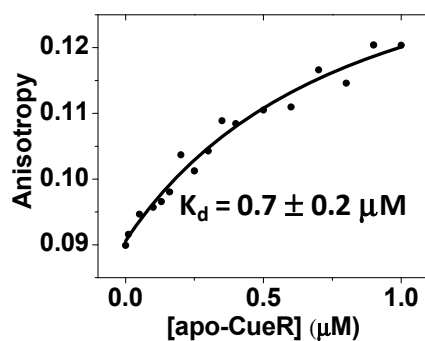


Figure S4. Fluorescence anisotropy experiment on Cy-3 labeled double-strand DNA containing only half of the dyad-symmetric sequence (5'-TGACCTTCCCCTTGGCTTGTT-3', the half sequence is underlined) titrated with apo-CueR. The solid line is the fit using Eq. 5 which gave a $K_D \sim 0.7 \mu\text{M}$.

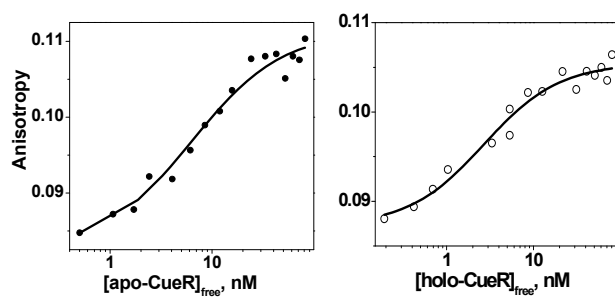


Figure S5. Data from Fig. 7 plotted against free protein concentrations.

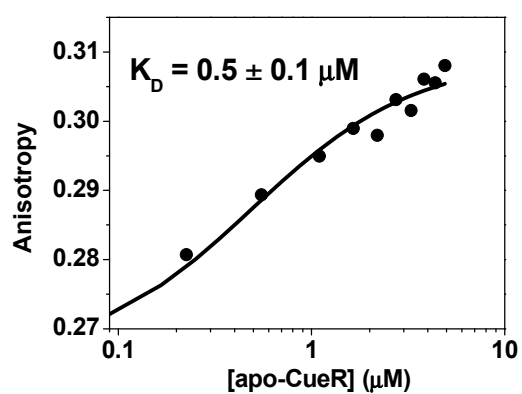


Figure S6. Fluorescence anisotropy experiment on Cy3-labeled HJC2 titrated with apo-CueR. The solid line is the fit using Eq. 5 giving a $K_D \sim 0.5 \mu\text{M}$ which is in between the affinity of apo-CueR to conf-I and to conf-II of HJC2 determined from single-molecule measurements.